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Genetic Risk Assessment and Specific-Locus Mutations in the *ad-3* Region of *Neurospora crassa*

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Data from experiments on the induction of specific-locus mutations in model systems are used in genetic risk assessment to estimate potential adverse effects in the human population. In such assessments with radiation or chemical mutagens, the following information is required: *a*) spontaneous and induced forward-mutation frequencies, *b*) dose–response curves for the overall induction of specific-locus mutations, c) genetic characterization of spontaneous and induced mutations, and *d*) dose–response curves for the different genotypic classes. Specific-locus assays in most eukaryote assay systems provide only portions of the information required for such assessments. In recognition of the need for more detailed information for risk assessment, a model system has been developed for specific-locus assays in *Neurospora crassa*. The adenine-3 *(ad-3)* specific-locus assay was modeled after the two-gene morphological specific-locus assay in the dilute-short-ear region of the mouse and detects forward-mutations at two closely linked loci: *ad-3A* and *ad-3B*. A computerized data management program has made it possible to obtain precise dose–response curves not only for the overall induction of *ad-3* mutations, but also for various genotypic subclasses. In addition, computerized statistical programs have been developed to compare dose–response curves. These methods of analysis have shown that the overall dose–response curve for specific-locus mutations in the *ad-3* region is a composite of many different genotypic subclasses. In addition, these subclasses may have very different induction kinetics from those of the overall dose–response curve for *ad-3* mutations.

Introduction

One objective in genetic risk assessment is to use specific-locus assays in model systems as a means of approximating the impact of successful transmission of genetic damage resulting from exposure to mutagenic environmental agents on F_1 progeny and subsequent generations in the human population. For these exercises, the model system of choice is the mouse (*1–3*). There are extensive data on the genetic effects of various radiations and chemical mutagens from studies with the morphological specific-locus assay system developed by Russell (*4*), which detects mutations at seven loci.

Mouse data have been exceptionally useful in genetic risk assessment exercises for many years. However, one of the limitations of this approach is the time and expense of the experiments required to collect such specific-locus mutations, as well as their subsequent genetic characterization (*5–7*). Because of the large populations of mice that would be required to provide enough data for any specific locus in the morphological specific-locus assay, dose–response curves combine the yields of mutations at all seven loci as a practical necessity. In addition, the resources and time required for genetic and molecular analysis of mutations at any given locus precludes the development of dose–response curves for individual genotypic classes (e.g., gene/point mutations vs. multilocus deletion mutations).

For genetic risk assessment, to determine if there is a significant increase in the frequency of specific-locus mutations after mutagenic treatment, it is essential to be able to compare spontaneous and induced forward-mutation frequencies. Estimates of both spontaneous and induced forward-mutation frequencies must be made with a high degree of precision, especially with low-level exposures to mutagenic agents. It is equally essential to know whether the induced spectrum of specific-locus mutations is qualitatively different from those that occur spontaneously. If the induced spectrum is qualitatively different from that occurring spontaneously, then the impact of successful transmission to F_1 progeny could be quite different in terms of heterozygous effects (*8–11*). The ability to obtain dose–response curves makes it possible to extrapolate from the high-dose treatments used in the laboratory on model systems to the low exposures that may occur in nature to the human population. Genetic characterization of specific-locus mutations is essential in determining the impact of successful transmission of induced mutations to F_1 progeny. It may also provide an understanding of the "ground rules" associated with heterozygous effects of specific-locus mutations resulting either from gene/point mutation or multilocus deletion mutations (*8–11*).

In recognition of the need for a specific-locus assay system that could provide the type of data required to permit a comprehensive genetic risk assessment, a new model system was developed with *Neurospora crassa* (*12–14*). The *ad-3* assay was modeled after the dilute-short-ear *(dse)* morphological specific-locus assay in the mouse, developed by Russell (*4*). In the mouse assay, two different types of morphological mutations can be detected in

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the *dse* region on chromosome 9 as changes in coat color and ear size. In some cases, double mutants are detected, usually as the result of deletion of both of these closely linked genes (*7*). It was possible to mimic this assay in Neurospora because there are two closely linked genes in the *ad-3* region, namely, *ad-3A* and *ad-3B*. Because *ad-3* mutations accumulate a reddish-purple pigment as well as having a requirement for adenine, it was possible to develop a direct method (*14*) for their recovery based on pigment accumulation. With this method, about 1.25×10^6 heterokaryotic survivors are incubated in 10 L of medium (in a 12-L Florence flask) supplemented with 12.5 mg/L adenine and 10 mg/L nicotinic acid. After about 7 days of incubation in the dark with aeration at 30°C, surviving conidia form colonies about 1–2 mm in diameter; colonies resulting from *ad-3* mutations are reddish-purple, and nonmutant colonies are colorless. Thus, the *ad-3* assay is both a morphological and a biochemical specific-locus assay system. The direct method has made it possible to recover from several hundred to several thousand *ad-3* mutations after mutagenic treatment, even at low frequencies of induction. This method also provides very accurate estimates of overall *ad-3* forward-mutation frequencies.

Typically, from 100 to 250 *ad-3* mutations are reserved for genetic characterization from each treatment series. The low frequency of spontaneous *ad-3* mutations of 0.39×10^{-6} survivors usually ensures that such mutations are a minor fraction of samples of induced *ad-3* mutations in most forward-mutation experiments. In addition, the collection of large numbers of mutants of spontaneous origin from successive experiments has made it possible to determine the mutational spectrum of spontaneous *ad-3* mutations (*15*).

Because of the efficacy of the *ad-3* specific-locus assay, the large amount of data that can be collected in a typical experiment provides precise dose–response curves for survival and the overall induction of *ad-3* mutations. The computerized data management program developed by Smith and de Serres (*16*) provides a useful mechanism for data storage, tabulation, and statistical analysis of the data from forward-mutation experiments. Subsequent data generated by the genetic analysis of samples of *ad-3* mutations from each treatment series make it possible to resolve the overall dose–response curve for *ad-3* mutations into its different genotypic components. Such data have provided striking evidence for mutagen specificity, both quantitative with regard to mutagenic potency and qualitative with regard to mutational spectra. These analyses have also shown that the major genotypic classes of *ad-3* mutations, and various subclasses, may have different induction kinetics. In addition, classes of mutations (e.g., multiple-locus *ad-3* mutations with closely linked recessive lethal mutations) that would not be expected to be recovered on the basis of target theory (*17*), have been found to occur at markedly higher frequencies than expected (*18–21*). Data from studies with various chemical mutagens have shown that some agents produce specific-locus mutations in the *ad-3* region predominantly or exclusively by gene/point mutation, whereas other agents produce both gene/point mutations and multilocus deletion mutations (*22*).

Computerized Data Management Program

A computerized data management program was developed by Smith and de Serres (*16*) to handle all aspects of the induction and genetic characterization of *ad-3* mutations. The first computer printout from each experiment lists all of the data from forward-mutation experiments and provides dose–response curves for the survival of the treated cell populations, and induction of *ad-3* mutations. These data are used to select at least three samples of *ad-3* mutations from different treatment series for genetic analysis. The second printout lists all the data from such genetic analyses (*23*) on individual *ad-3* mutations. These data are also collated into the three major genotypic classes: gene/point mutations, multilocus deletion mutations, and unknowns (mutations that grow too rapidly in the absence of adenine). The three major genotypic classes and five subclasses that can be detected with these analyses are listed in Table 1. In the second computer printout, the overall *ad-3* forward-mutation frequency is used to determine the frequency of mutants in each major class or subclass. The data from all treatments are then used to develop a series of dose–response curves for each of these major classes and subclasses.

The next stage of analysis in the computerized data management program makes it possible to compare and combine data. With these programs it is possible to combine data from different experiments and/or compare dose–response curves for different genotypic classes with regard to slope or displacement on a log–log plot. This data management program has been used extensively since its development in 1968 for a wide variety of experiments with both ionizing and nonionizing radiations and various chemical mutagens (*22*).

Genomic and Specific-Locus Assays that Provide a Database for Genetic Risk Assessment

In recognition of a need to assay the induction of recessive lethal mutations over the entire genome, Atwood and Mukai (*24,25*) developed a two-component heterokaryon of the haploid fungus *Neurospora crassa*. This approach was combined with the specific-locus assay approach in the development of the adenine-3 (*ad-3*) forward-mutation test in Neurospora by de Serres and co-workers (*12,14,23*).

In Neurospora, adenine-3A (*ad-3A*) and adenine-3B (*ad-3B*) also are closely linked (about 0.1 map unit), with closely linked markers located both proximally (histidine-3 [*his-3*], lysine-4 [*lys-4*], and histidine-2 [*his-2*]), and distally (nicotinic acid-2 [*nic-2*]). Also, there are 16 additional loci, with unknown biochemical requirements, that serve as markers in the *ad-3* and immediately adjacent regions (*26*). The *ad-3* assay system was designed to detect mutations occurring at *ad-3A* and *ad-3B* as well as at other loci in the immediately adjacent genetic regions. The recovery of such mutations was not expected, however, since they should occur at extremely low frequencies on the basis of target theory (*17*).

This assay is also unusual in that gene/point mutations can be readily distinguished from multilocus deletion mutations by a series of simple biochemical and genetic tests (*23*). For example, gene/point mutations resulting from intragenic alterations in either *ad-3A* or *ad-3B* can be compensated for by adding adenine to the basic minimal medium. Such mutations are "reparable" on such a medium, and are designated *ad-3*^{R}.

Table 1. Genotypic classes and subclasses of specific-locus mutations in the *ad-3* region that can be detected with two-component heterokaryons of *Neurospora crassa*.

Genotype	Description
Σ *ad-3*	All classes of *ad-3* mutations
Σ*ad-3*^{R}	All classes of gene/point mutations
ad-3A^{R} *ad-3B*^{R}	Gene/point mutations at the *ad-3A* or *ad-3B* locus with no known sites of genetic damage elsewhere in the genome
ad-3A^{R} + *RL*^{CL} *ad-3B*^{R} + *RL*^{CL}	Multiple-locus (*ad-3*)^{R} mutation: gene/point mutations at the *ad-3A* or *ad-3B* locus with a recessive lethal mutation in the immediately adjacent regions
ad-3A^{R} + *RL* *ad-3B*^{R} + *RL*	Multiple-locus (*ad-3*)^{R} mutation: gene/point mutations at the *ad-3A* or *ad-3B* locus with a recessive lethal mutation elsewhere in the genome.
Σ (*ad-3*)^{IR}	All classes of multilocus deletion mutations
(*ad-3A*)^{IR} (*ad-3B*)^{IR} (*ad-3A ad-3B*)^{IR} (*ad-3B nic-2*)^{IR} (*ad-3A ad-3B nic-2*)^{IR}	Multilocus deletion mutations covering the *ad-3A*, *ad-3B*, and/or *nic-2* loci
(*ad-3A*)^{IR} + *ad-3B*^{R} (*ad-3B*)^{IR} + *ad-3A*^{R} (*ad-3A*)^{IR} + *RL*^{CL} (*ad-3B*)^{IR} + *RL*^{CL}	Multiple-locus(*ad-3*)^{IR} mutation: multilocus deletion mutations covering the *ad-3A* and/or, *ad-3B* with a recessive lethal mutation in the mutation in the immediately adjacent regions
(*ad-3A*)^{IR} + *RL* (*ad-3B*)^{IR} + *RL* (*ad-3A ad-3B*)^{IR} + *RL* (*ad-3B nic-2*)^{IR} + *RL* (*ad-3A ad-3B nic-2*) + *RL*	Multiple-locus (*ad-3*)^{IR} mutation: this class is indistinguishable from multilocus deletion mutations covering the *ad-3A*, *ad-3B*, and/or *nic-2* loci with present strains used as testers, and can not be detected individually
Σ *ad-3*^{UNKN}	Pigmented isolates that grow too rapidly on minimal medium to be characterized.

However, multilocus deletion mutations that inactivate either one or both genes, as well as other genes in the immediately adjacent regions, cannot be compensated for by the addition of adenine or any other supplement to the basic minimal medium. Such mutations are "irreparable" on such media and are designated *ad-3*^{IR}.

Genetic Characterization of *ad-3* Mutations Using Classical Genetic Assays

Genetic characterization of *ad-3* mutants is made by a series of heterokaryon tests for complementation with nine tester strains to determine genotype (*ad-3A*, *ad-3B*, and/or *nic-2*), and allelic complementation among *ad-3B*^{R} mutants (*27–29*) to distinguish noncomplementing mutants from complementing with either nonpolarized or polarized complementation patterns. The results of such heterokaryon tests, and the testers used, are illustrated in de Serres (*30*). These heterokaryon tests are followed by *a*) dikaryon tests (to determine whether the newly induced *ad-3* mutant is capable of growing as a haploid homokaryon on medium supplemented with adenine (and pantothenic acid), or with adenine, nicotinic acid (and pantothenic acid), and *b*) trikaryon tests (with three strains carrying multilocus deletion mutations of various sizes in the *ad-3* and immediately adjacent regions). The three strains used as testers are 12-1-18 (*ad-3A ad-3B nic-2*)^{IR} (code no. 308), 12-7-215 (*ad-3A*)^{IR} (code no. 021), and 12-5-182 (*ad-3B*)^{IR} (code no. 038). For a more detailed description, see de Serres (*19*). The dikaryon and trikaryon tests permit a further characterization of those gene/point mutations and multilocus deletion mutations resulting from different types of multiple-locus mutations (*19,20*).

The different genotypes of *ad-3* mutants that can be described on the basis of the data collected in the three genetic tests used for characterization are given in Table 1. In this Table, the six genotypic subclasses expected by us initially (*12,13*) on the basis of target theory (*17*) are given. These genotypes consist of gene/point mutations at the *ad-3A* locus or the *ad-3B* locus, as well as multilocus deletion mutations covering one or both of these loci as well as the adjacent *nic-2* locus, located distally. An additional group of 49 genotypic subclasses that can be detected with this assay, many of which have actually been detected in various forward-mutation experiments, is discussed in de Serres (*22*).

Lessons from Studies with Radiation and Chemical Mutagens

Dose–Response Curves Have Demonstrated Striking Quantitative Differences in Mutagenic Potency

The dose–response curves for the induction of *ad-3* mutations after treatment with chemical mutagens (Fig. 1) have demonstrated marked differences in slope and mutagenic potency (*31*). The following classification scheme for mutagenic potency has been adopted: weak mutagens (1 – 10 *ad-3* mutations per 10^6 survivors), moderate mutagens (10 – 100 *ad-3* mutations per 10^6 survivors), strong mutagens (100 – 1,000 *ad-3* mutations per 10^6 survivors), and potent mutagens (1,000 to 10,000 *ad-3* mutations per 10^6 survivors). The weakest mutagen is 2-aminopurine (*32*), and the most potent ever detected with this assay system is 2-amino-N^6-hydroxyadenine (*33*).

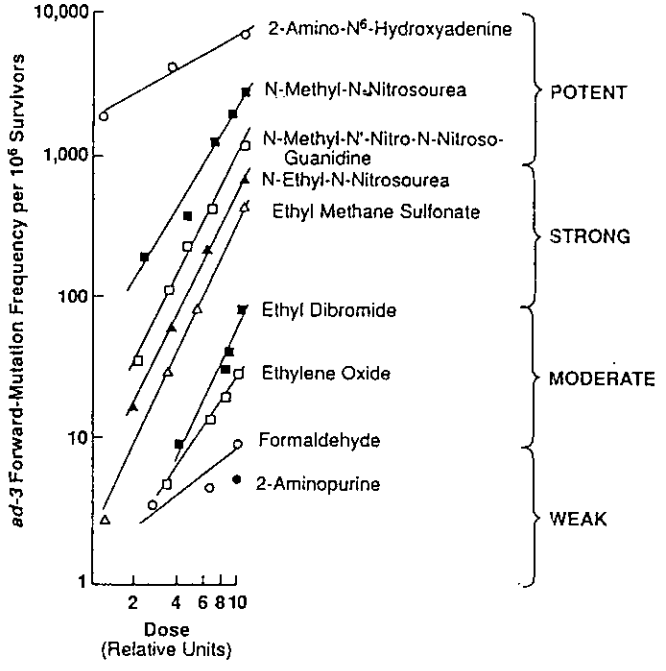

FIGURE 1. Potency comparisons of the dose–response curves for the induction of *ad-3* mutations by chemical mutations.

Overall Forward-Mutation Frequency for *ad-3* Mutations Can Result from Gene/Point Mutations and Not Multilocus Deletion Mutations

Experiments with the chemical mutagens procarbazine (*34*) and nitrous acid (*35*) have shown that the overall dose–response curve for *ad-3* mutations can consist solely of gene/point mutations (Σ *ad-3*^{R}) and not multilocus deletion mutations (Σ *ad-3*^{IR}). At least 12 additional chemicals (Table 2) fall into this same category (*22*). The low percentages of multilocus deletion mutations found in some samples (e.g., SQ18506) could be of spontaneous origin.

Overall Forward-Mutation Frequency for *ad-3* Mutations Can Result from Gene/Point Mutations and Multilocus Deletion Mutations

The data from additional experiments with chemical mutagens have shown that some chemicals induced specific-locus mutations by both gene/point mutations and multilocus deletion mutations. The fraction of *ad-3* mutations in each class can vary from 2.7 to 88.0% (Table 3).

Spectrum of *ad-3* Mutations Can Be Dose Dependent or Dose-Independent

Experiments with X-rays (*13,26,37*) have shown that X-ray-induced *ad-3* mutations result from both major classes, gene/point mutations and multilocus deletion motions, but that the percentages of *ad-3* mutations in each class is dose-dependent over a wide range of survivals and forward-mutation frequencies.

Experiments with ultraviolet light [UV (*36*)] have shown that UV-induced *ad-3* mutations also result from both gene/point mutations and multilocus deletion mutations. However, the percentages of *ad-3* mutations in each major class is dose-dependent over a comparable range of survivals and forward-mutation frequencies.

Association of *ad-3* Mutation with a Recessive Lethal Mutations Elsewhere in the Genome Varies According to Agent and Dose

The specific-locus *ad-3* assay system can detect recessive lethal mutations occurring outside of the *ad-3* region on linkage group I or on the other six linkage groups. Studies with X-rays (*21*) have shown that gene/point mutations with a recessive lethal elsewhere in the genome (*ad-3*^{R} + *RL*) increase as the square of X-ray dose; thus, such mutations are dose-dependent. At very high X-ray doses, the presence of additional sites of recessive lethal damage elsewhere in the genome must be taken into consideration in genetic risk assessment. Such damage could be transmitted to F_1 progeny.

Such *ad-3* mutations with additional sites of recessive lethal damage are also found in experiments with such chemicals as procarbazine (*34*) and 2-amino-N^6-hydroxyadenine (*33*), and the frequencies of such mutations are also dose-dependent. In the latter case, from 3 to 70% of *ad-3* gene/point mutations can have a recessive lethal mutation elsewhere in the genome (de Serres and Brockman, unpublished data). The presence of additional sites of recessive lethal damage elsewhere in the genome in specific-locus mutations is important for extrapolation of data on their induction to overall risk to the genome. If the frequency of such multiple-locus mutations is high, then genetic risk could be grossly underestimated.

Frequency of Multiple-Locus Mutations with Closely Linked Recessive Lethal Mutations Is Higher Than Expected on Target Theory

It was assumed that multiple-locus mutations, especially those resulting from mutations in closely linked genes, such as *ad-3A* and *ad-3B*, would be the product of their individual frequencies. Thus, if the forward-mutation frequency for an X-ray-induced *ad-3A* mutant was 1×10^{-6} and an *ad-3B* mutant was 2×10^{-6}, the frequency of X-ray induced *ad-3A ad-3B* double mutants should be 2×10^{-12}. Two different classes of multiple-locus mutation with closely linked recessive lethal mutations can be distinguished (Table 1). Either gene/point mutations or multilocus deletion mutations can occur in combination with closely linked recessive lethal mutations (*ad-3*^{R} + *RL* ^{CL} or *ad-3*^{IR} + *RL*^{CL}).

In our experiments with X-ays, for example, such multiple-locus mutations were found to occur at much higher frequencies (*18–21,30*) than expected on the basis of target theory (*17*). The large numbers of such mutants recovered with the *ad-3* forward-mutation assay have provided sufficient data to investigate the induction kinetics of these two unexpected subclasses of *ad-3*

Table 2. Chemicals that include ad-3 mutants predominantly, or exclusively, by gene/point mutation.

Experiment Number	Mutagen	Gene/point Mutations		Multilocus deletion mutations		Reference
		No.	%	No.	%	
12-009	ICR-170	187	100.0	0	0.0	(*49*)
12-196	4NQ0	184	100.0	0	0.0	(*50*)
12-197	4HAQ0	210	100.0	0	0.0	(*50*)
12-314	NDZ	199	100.0	0	0.0	(*51*)
12-683	PROCARB	208	100.0	0	0.0	(*34*)
12-004	NA	417	99.8	1	0.2	(*35*)
12-027	MNNG	953	99.4	6	0.6	(*52*)
12-267	AF-2	262	99.2	2	0.8	(*53*)
12-163	ENU	218	99.1	2	0.9	(*39*)
12-021	HA	202	99.0	2	1.0	(*54*)
12-028	DEN	92	98.9	1	1.1	(*55*)
12-265	FANFT	213	98.6	3	1.4	(*53*)
12-264	SQ18506	212	98.6	3	1.4	(*53*)
12-315	MTZ	139	98.1	3	1.9	(*51*)

Abbreviations: NA, nitrous acid; ICR-170, 2-methoxyhloro-9-[3-(ethyl-2-chlorethyl)aminopropylamino] acridine dihydrochloride; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; HA, hydroxylamine; DEN, diethylnitrosamine; 4NQ0, 4-nitroquinoline 1-oxide; 4HAQ0, 4-hydroxyaminoquinoline 1-oxide; NDZ, niridazole; MTZ, metronidazole; AF-2, 2(2-furyl)-3-(5-nitro-2-furyl)acrylamide; SQ18506, trans-5-amino-3-[2-(5-nitro-2-furyl)-vinyl-1,2,4-oxadiazole; FANFT, 2-formylamino-4-(5-nitro-2-furyl) thiazole; ENU, ethylnitrosourea; PROCARB, procarbazine.

Table 3. Chemicals that induce ad-3 mutants by both gene/point mutation and multilocus deletion mutation.

Experiment number	Mutagen	Gene/point mutations		Multilocus deletion mutations		Reference
		No.	%	No.	%	
12-244	IA-5	11	12.0	81	88	(*56*)
12-254	IA-4	16	12.9	108	87.1	(*56*)
12-250	LUC	11	16.9	54	83.1	(*56*)
12-243	HYC	19	17.8	88	82.2	(*56*)
12-248	IA-3	15	18.5	66	81.5	(*56*)
12-217	DE0	162	70.4	68	29.6	(*57*)
12-194	PDMT	45	80.3	11	19.6	(*58*)
12-225	EDB	327	84.3	61	15.7	(*31*)
12-650	2AP	203	87.9	28	12.1	(*32*)
12-199	DEP	201	93.1	15	6.9	(*57*)
12-189	PMMT	170	94.4	10	5.6	(*58*)
12-064	MMS	501	94.5	29	5.5	(*59*)
12-180	EI	180	97.3	5	2.7	(*60*)

Abbreviations: MMS, methylmethanesulfonate; EI, ethylenimine; PMMT, 1-phenyl-3-monomethyltriazene; PDMT, 1-phenyl-3-dimethyltriazene; DEP, 1,2,4,5-diepoxypentane; DE0, 1,2,7,8-diepoxyoctane; HYC, hycanthone methanesulfonate; LUC, leucanthone hydrochloride; IA-3, hycanthone methanesulfonate indazole analog; IA-4, leucanthone methanesulfonate indazole analog; IA-5, hycanthone methanesulfonate indazole analog; EDB, ethylene dibromide; 2AP, 2-aminopurine.

mutations (*21*). Both classes increase as the square of X-ray dose. These data are somewhat surprising because on the basis of target theory, some of the *ad-3* mutations with multiple sites of recessive lethal damage would require from three to eight events to account for their induction.

These findings must also be taken into account in genetic risk assessment because there is a high probability (approaching 20% at high X-ray doses) that specific-locus mutations will have at least one closely linked site of recessive lethal damage. Unexpectedly high frequencies of multiple-locus mutations with closely linked recessive lethal mutations have also been found in our experiments with ethylene dibromide (*31*), 2-aminopurine (*32*), and 2-amino-N^6-hydroxyadenine (*33*), but not with procarbazine (*34*).

Results parallel to those found in Neurospora also have been found in *in vivo* germinal specific-locus systems in both the mouse and Drosophila. In the mouse, multiple-locus mutations with closely linked sites of damage were identified by Russell and Rinchik (*7*) among X-ray-induced specific-locus mutations in the *dse* region. These mutations were designated "skipping mutations." In Drosophila, multiple-locus mutations were found among X-ray-induced specific-locus mutations at the white locus in the repair-deficient strain *mus-201* (*38*). In both organisms, the "exceptional" mutants are similar to the X-ray-induced multiple-locus $(ad\text{-}3)^{IR}$ mutations in Neurospora.

The high frequency of such multiple-locus mutations indicates a much higher, and more extensive, type of genetic damage occurring at specific loci than was originally anticipated on the basis of target theory (*17*). Again, these data provide evidence for more complex and extensive genetic damage at specific loci that must be taken into account in genetic risk assessment. In addition, the frequency and type of such multiple-locus mutations may well vary as a function of mutagenic origin.

Spectrum of Multilocus Deletion Mutations in the *ad-3* Region Is Mutagen Dependent

Comparisons between the *ad-3* mutations classified as multilocus deletion mutations have revealed marked differences as a function of mutagenic origin (*31,39*). The five different genotypic classes can be ranked as a function of size as follows; $(ad\text{-}3A)^{IR}$, $(ad\text{-}3B)^{IR}$, $(ad\text{-}3A\ ad\text{-}3B)^{IR}$, $(ad\text{-}3B\ nic\text{-})^{IR}$, (*ad-3A*

Table 4. Comparison of the percentages of *ad-3* mutants resulting from gene/point mutations (*ad-3*^{R}) and multilocus deletions ([*ad-3*]^{IR}) in spontaneous and induced samples.

	SP		MMS		EDB		DEO		HYC	
Genotype	No.	%	No.	%	No.	%	No.	%	No.	%
Σ *ad-3*^{R}	166	100.0	530	100.0	387	100.0	162	100.0	107	100.0
Σ*ad-3*^{R}	141	84.9	501	94.5	327	84.5	94	58.0	19	17.8
ad-3A^{R}	41	24.7	182	34.3	103	26.6	35	21.6	7	6.5
ad-3B^{R}	100	60.2	319	60.2	224	57.9	59	36.4	12	11.2
Σ *(ad−3)*^{IR}	25	15.1	29	5.5	60	15.5	68	42.0	88	82.2
(ad−3A)^{IR}	6	3.6	4	0.8	16	4.1	9	5.9	1	0.9
(ad−3B)^{IR}	15	9.0	16	3.0	8	2.1	26	16.1	6	5.6
(ad-3A ad-3B)^{IR}	4	2.4	8	1.5	32	8.2	22	14.4	3	2.8
(ad-3B nic-2)^{IR}	0	0.0	0	0.0	0	0.0	2	1.3	9	8.4
(ad-3A ad-3B nic-2)^{IR}	0	0.0	1	0.2	4	1.0	9	5.9	69	64.5

Abbreviations: SP, spontaneous (0.39×10^{-6} S); MMS, methylmethanesulfonate [Σ of 13.3 to 366.7×10^{-6} S (*59*)]; EDB, ethylene dibromide [19.3×10^{-6} S (*31*)]; DEO, 1,2,7,8-diepoxyoctane [50.1×10^{-6} S (*57*)]; HYC, hycanthone methanseulfonate [35.0×10^{-6} S (*56*)].

ad-3B nic-2)^{IR}. In Table 4, samples of *ad-3* mutations induced by methyl methanesulfonate (MMS), ethylene dibromide (EDB), 1,2,7,8,-diepoxyoctane (DEO), and hycanthone methanesulfonate (HYC) are compared with those of spontaneous (SP) origin. In addition, our earlier studies (*31*) showed that X-ray-induced multilocus deletion mutations are larger than UV-induced multilocus deletion mutations.

These data show differences in the percentages of both radiation and chemically induced *ad-3* mutations resulting from multilocus deletion mutations as well as marked differences in the size of such mutations. It is particularly interesting that 78.4% (69/88) of the HYC-induced multilocus deletion mutations are in the largest size class in contrast to MMS, which has only 3.4% (1/29) in this class.

The conclusion that can be drawn from such data is that different mutagens not only produce varying frequencies of specific-locus mutations resulting from multilocus deletion mutations but that there are marked differences between their sizes.

Modification of Traditional Methods of Genetic Risk Assessment

The traditional methods of genetic risk assessment for ionizing radiation-induction mutations have been reviewed by Sankaranarayanan (*40*). These methods were originally developed to make maximum use of limited data from whole-animal model systems. There were inadequate data on both spontaneous and induced forward-mutation frequencies as well as on the spectra of spontaneous and induced mutation in particular genes.

The most widely used model system for human genetic risk assessment is the mouse. However, numbers of mice must be screened to obtain specific-locus mutations with either the morphological (*4*) or the biochemical (*41*) specific-locus assay. As a result, it is necessary to use data for the induction of forward-mutations at many loci, rather than at individual loci, to obtain dose–response curves after radiation treatment. Because there are limited data on mutation-induction at any given specific locus, it is generally assumed that the spectrum of specific-locus mutation is dose independent. It is also assumed, in the lack of sufficiently large samples of spontaneous and induced mutants, that the mutational spectra are identical. As a result, the doubling dose method (the amount of radiation required to produce as many mutations as those occurring spontaneously) is used to provide a mechanism for estimation of human genetic risk (*42*).

Data from forward-mutation experiments with the *ad-3* specific-locus assay can be used to supplement data from experiments with the mouse and to develop a more comprehensive approach to genetic risk assessment. The data on spontaneous *ad-3* forward-mutations (Table 4) indicate that this spectrum is not only clearly different from those *ad-3* mutations induced by MMS, EDB, DEO, and HYC, but also from X-ray-induced mutations (*26,37*). In addition, Webber and de Serres (*13*) demonstrated that the spectrum of *ad-3* forward-mutations is dose dependent. These observations have been confirmed and extended in more recent studies (*26,37*), all of which have shown that gene/point mutations increase linearly with X-ray dose, whereas multilocus deletion mutations and multiple-locus mutations increase as the square of X-ray dose.

It is also generally assumed (*1–3*) that most X-ray-induced specific-locus mutations will be recessive. However, X-ray-induced multilocus deletion mutations in the dilute-short-ear region of the mouse were demonstrated to show striking, allele-specific heterozygous effects (*43*). Similar data were presented from studies of X-ray-induced *ad-3A* or *ad-3B* mutants resulting from multilocus deletion (*44,45*).

Recent studies with X-ray-induced multilocus deletions in Drosophila (*46*) have also shown allele-specific heterozygous effects. The conclusion that can be drawn from these studies on experimental organisms is that alleles of genes expected to show recessive Mendelian inheritance may show partial dominance in terms of heterozygous effects affecting growth rate, viability, longevity, etc. Thus, past assumptions of the ratio of dominant to recessive mutations that can be expected after exposure to radiation (*1–3*) may be in error, and this ratio may well vary as a function of mutagenic agent. Furthermore, if the spectrum (e.g., ratio of gene/point mutations to multilocus deletion mutations) of induced specific-locus mutations is dose dependent, the ratio of dominant to recessive mutations could vary as a function of forward-mutation frequency. Little is known about the mechanisms of such allele-specific heterozygous effects from experiments with specific-locus mutations in the mouse or Drosophila and whether these effects are confined to multilocus

deletion mutations. However, experiments with X-ray-induced *ad-3* mutations resulting from gene/point mutation as well as those resulting from multilocus deletion mutation or multiple-locus mutation have demonstrated allele-specific heterozygous effects on linear growth rate in Neurospora (*11,47,48*).

In conclusion, it is clear that much additional work must be done to investigate the impact of successful transmission of both spontaneous and induced mutations to the F_1 and subsequent generations both in the mouse and Drosophila. The exploratory studies on the heterozygous effects of X-ray-induced specific-locus mutations in Drosophila (*46*), mouse (*7,43*), and Neurospora (*44,45*) indicate that dominance/recessiveness is allele specific rather than gene specific. If heterozygous effects are also mutagen dependent, then all of these organisms must be used to further explore such effects and to obtain an understanding of any differences in the behavior of mutants induced by different mutagens.

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